

this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

***Amendments***

***In the Specification:***

*Please insert the sequence listing at the end of the application.*

*Please replace the paragraph beginning on page 1, line 2 with the following paragraph:*

The present application is a continuation in part of 08/347,610, filed December 1, 1994, which is a continuation in part of U.S. Patent Application Ser. No. 08/159,339, filed Nov. 29, 1993, now U.S. Patent 6,037,135, which is a continuation in part of U.S. Patent Application Ser. No. 08/103,396, filed Aug. 6, 1993 (now abandoned), which is a continuation in part of U.S. Patent Application Ser. No. 08/027,746, filed Mar. 5, 1993 (now abandoned), which is a continuation in part of U.S. Patent Application Ser. No. 07/926,666, filed Aug. 7, 1992 (now abandoned). The present application is also related to U.S. Patent Application Ser. No. 08/186,266, filed January 25, 1994, now U.S. Patent 5,662,907, which is a continuation in part of U.S. Patent Application Ser. No. 08/159,339 as described above.

All of the above applications and patents are hereby incorporated by reference as if fully set forth.

*Please replace the paragraph beginning on page 55, line 12 with the following paragraph:*

As a radiolabeled probe, the peptide 941.12 (KVFPYALINK) (SEQ ID NO:52), containing an A3.2 motif, was used. This peptide contains the anchor residues V<sub>2</sub> and K<sub>10</sub>, associated with A3.2-specific binders, described above. A Y residue was inserted at position 5 to allow for radioiodination. Peptides were labeled by the use of the Chloramine T method, Buus *et al.*, Science 235:1352 (1987), which is incorporated herein by reference.

*Please replace the paragraph beginning on page 57, line 11 with the following paragraph:*

The cell line BVR was used as a source of HLA. The dependency of the binding on MHC concentration in presence or absence of  $\beta_2$ M are shown in Fig. 6, while Fig. 7 depicts the dose dependency of the inhibition by excess unlabeled ligand. Finally, Fig. 8 shows a Scatchard analysis experiment. Values of apparent kD of -6 nM and of 10% active receptor were obtained, and were remarkable for their similarity to the values obtained for A2.1 and A3.2. The sequence of the peptide used as a radiolabeled probe (940-06) is AVDLYHFLK (SEQ ID NO:53).

*Please replace the paragraph beginning on page 57, line 22 with the following paragraph:*

In this case, the EBV cell line Steinlin was used as a source of purified HLA. The same protocol previously applied to purification of other HLA alleles (i.e., depletion of B, C molecules by a B1.23.2 mAb column, followed by purification of A molecules by means

of a W632 mAb column) was utilized. On the basis of the pool sequencing data, consensus peptides were synthesized, directly radiolabeled, and tested for HLA binding using the standard protocol (1 mM  $\beta_2$ M, 2 days RT incubation in presence of protease inhibitors). A graph illustrating the relationship between % binding and  $\mu$ M input HLA A1 is shown in Fig. 9. From the data, it was concluded that in analogy with what was observed for HLA A2, 3, and 11, as little as 30 nM are sufficient to obtain ~10% binding. The sequence of the peptide used as a radiolabeled probe (944.02) is YLEPAIAKY (SEQ ID NO:54). In the next set of experiments, the specificity of the assay established was verified by its inhibitability by excess unlabeled peptide. The IC50% was measured (Fig. 10) as ~20 nM. Further Scatchard analysis (Fig. 11) verified that the apparent  $K_D$  of the interaction corresponded to 21 nM, with a % of active receptor corresponding to 5.1%.

*Please replace the paragraph beginning on page 58, line 6 with the following paragraph:*

HLA A24 molecules were purified from the KT3 EBV cell line. In this case, two consensus peptides whose sequences were based on the pool sequencing data have been synthesized. Their sequences are: 979-01, AYIDNVYKF (SEQ ID NO:55) and 979.02, AYIDNLYNKF (SEQ ID NO:56). The results of experiments in which the % bound of these two peptides as a function of input MHC was measured are shown in Fig. 12. In both cases, 10-15% binding was obtained with as little as 20-50 nM MHC. Cold inhibition experiments (Fig. 13), limiting MHC concentrations, revealed that the binding was readily inhibitable by excess unlabeled peptide, with an apparent  $K_D$  of 30 and 60 nM, respectively. Further Scatchard experiments verified values of 136 nM and 28 nM, respectively. The apparent %

of available receptor (active MHC) were 8.3% and 7.4%, respectively (Fig. 9a and b). On the basis of these data, peptide 979.02 was arbitrarily selected as standard label indicator for A24 assays. Furthermore, on the basis of the data herein described, we also conclude that the goal of establishing an A24-specific binding assay has been accomplished. In conclusion, specific assays for the five major HLA alleles have been described.

*Please replace the paragraph beginning on page 59, line 5 with the following paragraph:*

For example, in the case of A3.2, a motif has been defined with a hydrophobic residue in position 2 and a positive charge (K) in position 9. Thus, to verify that the presence of these two anchor residues would allow, in the context of a poly A backbone, for A3.2 binding, the poly A analog with the sequence AMAAAAAAK (SEQ ID NO:61) was synthesized (Table 13).

*Please replace the paragraph beginning of page 65, line 30 with the following paragraph:*

Table 20(b) describes the peptides that bound to HLA-A3.2 molecules. Seven peptides were identified as high affinity binders, 6 as intermediate affinity binders and 13 as low affinity binders. Table 20(c) describes the peptides that bound to HLA-A11.2 molecules. Six high affinity peptides were identified, 4 intermediate affinity binders and 10 low affinity binders. Two high affinity binding peptides (E6-59 IVYRDGNPY (SEQ ID NO:142) and E6-80 ISEYRHYAY (SEQ ID NO:132)) and two weak affinity binding peptides with a Y at the 9th position (E6-42 QQLLRREVY (SEQ ID NO:155), E6-69

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VADKALKFY (SEQ ID NO:133)) were identified for HLA-A11.2. Considering the high binding strength of the first two peptides and the similarity between the HLA-A11.2 motif and the HLA-A3.2 motif in which Y's are preferred at the 9th aa position, tyrosines should be included at the 9th position in the HLA-A11.2 motif. Comparing Tables 21(b) and (c) it is clear that there is a large overlap of peptides that bound to both A3.2 and A11.2 molecules. Eighteen out of 28 E6 and E7 peptides binding to these two HLA molecules overlapped and only 8 peptides were unique for HLA-A3.2 and 2 peptides unique for HLA-A11.2.

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*Please replace the paragraph beginning on page 66, line 11 with the following paragraph:*

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Finally, Table 20(d) describes the peptides that bound to HLA-A24 molecules. Here 2 peptides were identified as high affinity binding peptides, 5 as intermediate affinity binding peptides and 5 as low binding peptides. One high affinity peptide (E6-72 KALKFYSKI) (SEQ ID NO:168) and one intermediate affinity peptide (E7-49 RAHYNIVTF) (SEQ ID NO:170) were identified, indicating that an A at the second position should be allowed in the HLA-A24 motif. All these inclusions are indicated in Table 20-e. In analyzing these tables it can be concluded that between 2 and 7 high affinity binding peptides were identified for all of the tested HLA-A molecules. Occasionally some peptides were binding to more alleles. Three peptides (E6-7, E6-37 and E6-79), bound to HLA-A2.1, A3.2 and A11.2. One peptide (E6-38) bound to HLA-A3.2, A11.2 and A24 and two peptides (E6-69 and E6-80) bound to HLA-A1, A3.2 and A11.2. But these crossreactive peptides bound only weakly to one or more of the different HLA molecules.

*as Contd*

In general, however, it can be concluded that, except for HLA-A3.2 and HLA-A11.2 molecules, almost all HLA molecules bind unique peptides.

*Please replace the paragraph beginning on page 87, line 34 with the following paragraph:*

*b9*  
All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

*b10*

*Please replace pending pages 50-54 with the attached substitute pages 50-54.*

*b11*

*Please replace pending pages 88-97 with the attached substitute pages 88-97.*

*b12*

*Please replace pending pages 103-117 with the attached substitute pages 103-117.*